~~R. J. Young~~

BIOPHYSICAL RESEARCH LABORATORY

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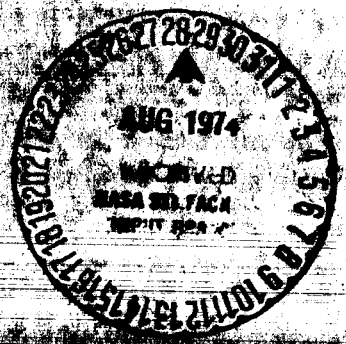
THIRTEENTH ANNUAL REPORT

Dr. Richard S. Young



CARNEGIE-MELLON UNIVERSITY
PITTSBURGH, PENNSYLVANIA

Jerome J. Wolken

March 28, 1968

BIOPHYSICAL RESEARCH LABORATORY

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I. INTRODUCTION

The Biophysical Research Laboratory was established at the Eye and Ear Hospital, University of Pittsburgh Medical Center, by grants from the McClintic Foundation in 1953. After a decade of research in the Medical Center, the Biophysical Research Laboratory moved to Carnegie Institute of Technology, Carnegie-Mellon University, to help strengthen the Biological Sciences in the College of Engineering and Science. The Laboratory continues to collaborate with the Eye and Ear Hospital. In 1964 new laboratories for the Biophysical Research Laboratory were built and equipped by a grant to Dr. Wolken from the Kresge Foundation and by grants from the Scaife Family and the Rachel Mellon Walton Foundation to carry on the research program of the Laboratory.

The research of this Laboratory for the past 14 years has been devoted to studies in photobiology, that is, how light, the visible part of the electromagnetic spectrum of energy, affects the behavior of molecules, cells and whole organisms. More specifically, our research has been directed primarily to attempts to understand the processes of photosynthesis and vision.

Research now in progress includes continued studies of these processes, that is, how living cells are able to capture light energy and to store or convert it to another form of energy such as from the absorption of light energy to ⟶ mechanical ⟶ chemical ⟶ electrical energy. This occurs in photosynthesis, vision, nervous excitation and other photobehavior.

In all responses to visible light by living organisms a group of yellow to orange to red pigment molecules is involved; these are carotenoids. In the plant world, they exist as a long polyene chain, consisting of forty carbon atoms, C_{40}, most familiar of which is a β-carotene. In the animal world, this chain of forty carbon atoms is split in half to a chain of twenty carbon atoms, C_{20}, which is familiar as vitamin A, necessary to the life of all animals. Somewhere in the scheme of evolution, animal cells evolved ways in which the forty carbon atoms are split into twenty; hence, the probable evolution from the plant ⟶ animal, C_{40} (β-carotene) ⟶ C_{20} (vitamin A).

To efficiently capture light, photoreceptor structures have evolved which are organized in molecular dimensions like a crystal - a lattice of ordered repeat units of about 100 Å spacing. What is important then is how chlorophyll and the carotenoids in the highly ordered chloroplasts function in photosynthesis; and how retinal, a carotenoid, in the highly ordered retinal rods and cones, functions in vision.

There are very special conditions under which life has been initiated - conditions which have made life and evolution possible. Nature is telling us something about all light-catalyzed reactions; therefore, we are searching carefully into the subtleties of these processes in order to understand them.

In the Annual Reports of the Biophysical Research Laboratory I-XIII (1953-1967), our year by year experimental findings are summarized. The Annual Reports also indicate the kind of laboratory,

the facilities, the personnel and the environment in which the research is carried out. Finally, these Reports express the excitement of our research, the disappointments and our hoped-for aims.

II. BIOPHYSICAL RESEARCH LABORATORY STAFF

Jerome J. Wolken	B.S., M.S. and Ph.D., University of Pittsburgh; Director, Biophysical Research Laboratory; Professor of Biophysics.
Gerald J. Gallik	B.S., University of Pittsburgh; Research Biologist.
Robert G. Florida	B.S., University of Pittsburgh; Research Assistant.
William R. Davis	B.S., Case Institute of Technology; M.S., University of Pittsburgh; Research Assistant.
Robert A. Cornesky	B.S., Geneva College; M.S., The George Washington University; Research Biologist.
George E. Marak, Jr.	B.A., Harvard University; M.D., University of Pittsburgh; U.S. Public Health Service Research Fellow.
Barbara S. Beals	B.S., Ph.D., University of Kentucky; Research Fellow.
Mahendra R. Vora	B.Sc., M.Sc., Ph.D., University of Bombay; Research Fellow.
Julie A. Klein	B.S.J., Ohio University; Secretary-Editorial Assistant.
Oliver J. Bashor	Laboratory Assistant.
Marjorie D. Spenser*	B.A., Antioch College; Research Assistant.
Grace Runnette Clark*	B.A., Mount Mercy College; Secretary.
Barbara Runnette*	Technician.

* Terminated

A. Part-time Associates

Robert Forsberg	B.S., Westminster College; Research Assistant.
Penn Lupovitch	B.S., Johns Hopkins; M.D., Chicago Medical College; Resident in Pathology, Montefiore Hospital.
Herbert Reitboeck	B.S., M.S. and Ph.D., Vienna Institute of Technology; Ph.D., University of Frankfurt; Westinghouse Research and Development Center, Senior Engineer.

B. Graduate Student Assistants

Kap Shik Ahn	B.S., M.S., Pusan National University; Chemistry, biology.
Robert Rothstein	B.A., University of Vermont; M.S., Columbia University; Biotechnology.

C. Summer Student Assistants

Auerbach, Anthony	Cornell University
Horn, Craig	Waynesburg College
Kogut, David	Johns Hopkins University
Lubiniecki, Anthony	Carnegie-Mellon University
Neil, Barbara L.	Carnegie-Mellon University
Wolken, A. Jonathan	Dartmouth College
Ellis, Norman	NSF Pre-College Student.
Tumpson, Daniel	NSF Pre-College Student.

III. RESEARCH PROGRESS

Research efforts in the past year have been directed to continued attempts to understand the process of visual excitation. These studies include the structure, biochemistry and electrophysiology of the retinal photoreceptors of the eye. In particular, studies included: (a) the optical systems of arthropod imaging compound eyes; (b) the structure of insect compound eyes; (c) the isolation of photosensitive pigments from compound eyes and their physical-chemical properties; and (d) information processing by compound eyes. Our research in photosynthesis was concerned with how the chloroplast pigments function in light excitation and with model molecular systems for the chloroplast. Concurrent with these studies was the completion of the Microspectrophotometer M-5 and the development of new instrumentation.

A. Studies of the Visual Process

1. Compound Eyes (Wolken)

The invertebrates, which include protozoa, coelenterates, flatworms, arthropods and molluscs, possess diverse eyes. Arthropods comprise the major group of invertebrates and include insects, arachnids and crustacea that possess compound eyes.

Over the years (refer to Annual Reports of the Biophysical Research Laboratory II-XII, 1955-1966) we have been investigating the structure and pigments of compound eyes in searching for understanding of the visual process.

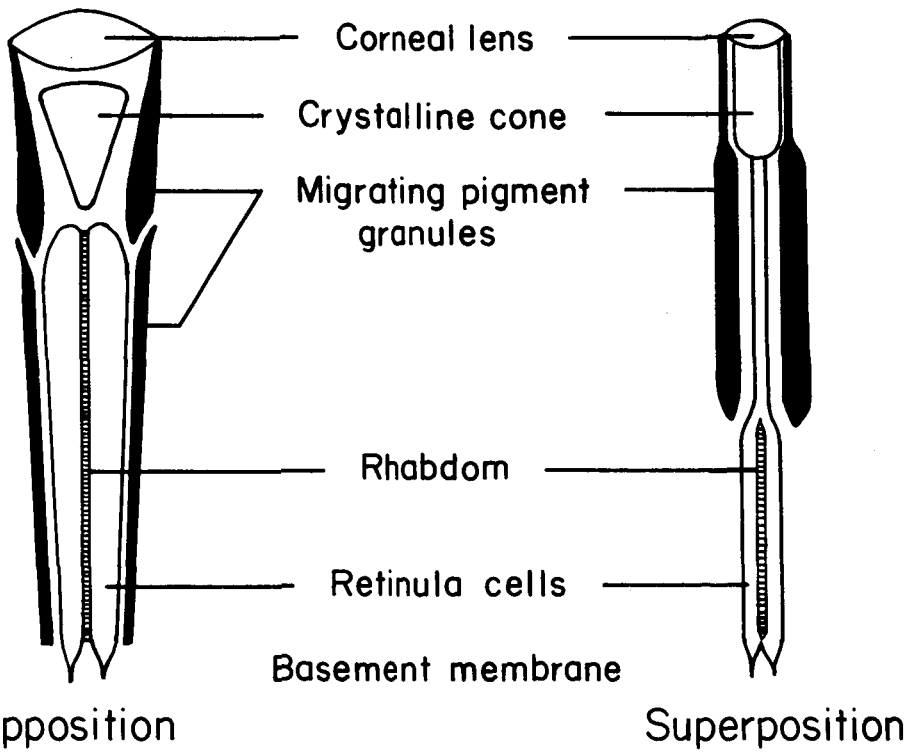


Figure 1 Schematics of two types of ommatidia.

The compound eye consists of eye facets, ommatidia, which vary in number from a few to as many as thousands. Each ommatidium has a corneal lens, crystalline cone and retinula cells that form its rhabdom, the retina (Figure 1). Because the corneal lens structure and light transmission are so important to determining how the optics and the visual system of compound eyes function, we have begun to fit together the structure, the optics, and the absorption and transmission of the various parts of the compound eye ommatidium. See, for example, the corneal lens of the firefly (Figure 2) and the light transmission spectrum of the housefly corneal lens (Figure 3). Each retinula cell has a specialized structure, the rhabdomere, which is analogous in function to the retinal rods of

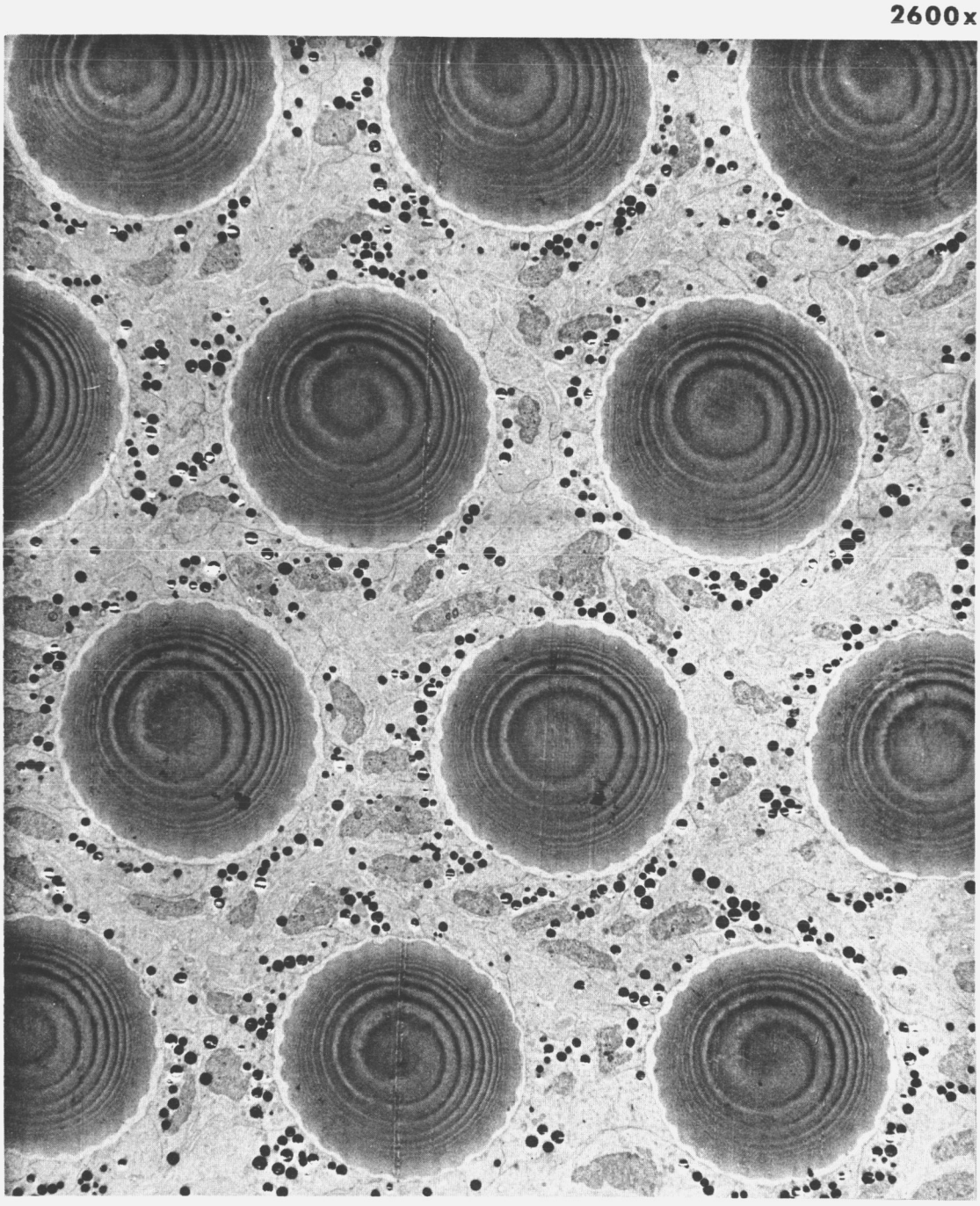


Figure 2 Corneal lenses of the firefly (Photuris pennsylvanica).

the vertebrate eye. Electron microscopy of the rhabdom of many arthropods shows that there are two kinds of geometry for the rhabdom; one is a "closed-type" (Figure 4) in which the rhabdomeres that form the rhabdom are in close proximity or are fused and the other is an "open-type" in which the rhabdomeres project into a cavity (Figure 5).

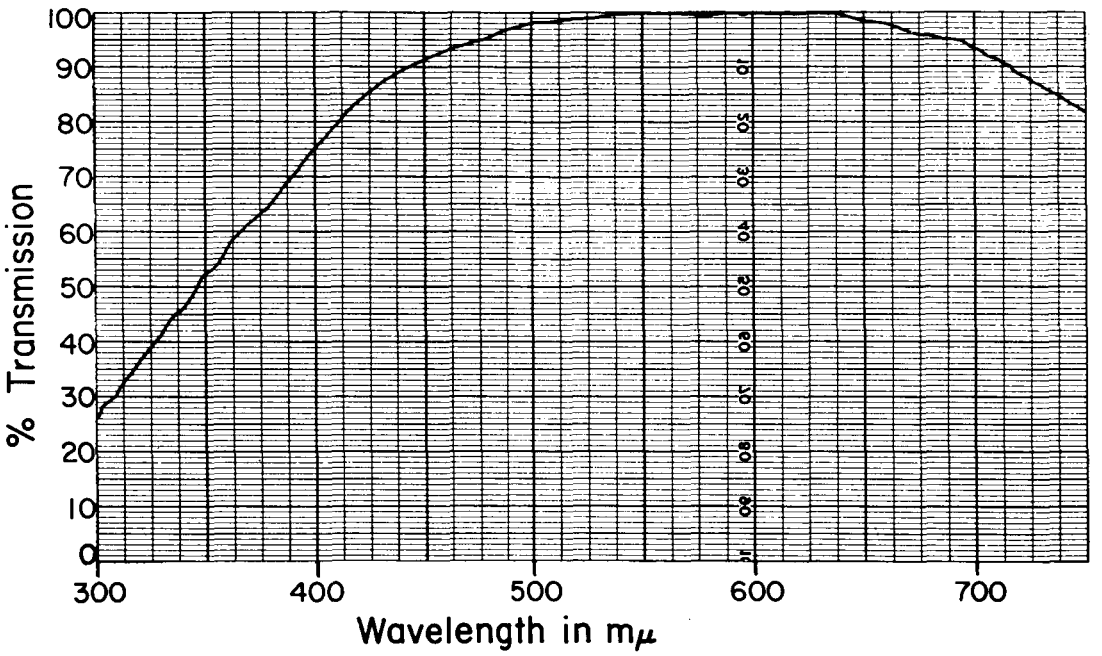


Figure 3 Transmission spectrum of the corneal lens of the fruitfly (Drosophila melanogaster, white-eyed mutant). (Microspectrophotometer M-5)

Exner, in 1891, described two anatomically distinct types of compound eyes, the apposition and the superposition. Apposition eyes are those in which the rhabdom lies directly against the crystalline cone (Figure 1a) and superposition eyes are those in which the rhabdom lies some distance away from the crystalline cone (Figure 1b).

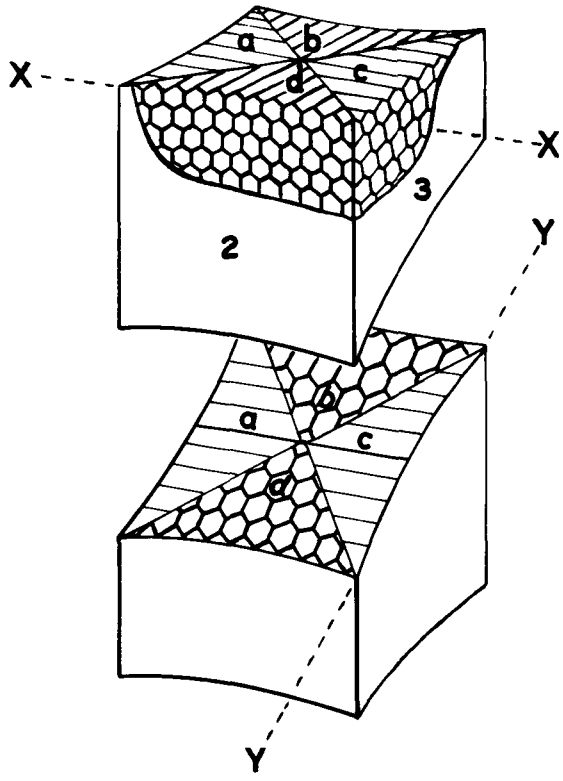


Figure 4 Rhabdom, fused type, showing how the structure depends on the angle of cut.

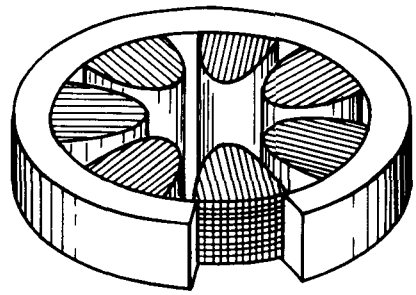


Figure 5 Rhabdom, open type. (cross section)

2. Electron Microscopy of Insect Compound Eyes (Marak and Gallik)

During the past year the structure of the compound eyes of the hornet, carpenter ant, firefly, clothes moth, cockroach and bee were studied by electron microscopy.

Carpenter Ant. The carpenter ant (Camponotus herculenus pennsylvanicus) has an apposition type eye that appears to be different from other hymenopteran eyes. There are only six retinula cells that contribute to a fused rhabdom of six distinguishable rhabdomeres. Each retinula cell ends in a long slender axonal process that travels through perforations in the basement membrane to form complex synapses with second order neurons. The rhabdom is circular and occupies a much larger proportion of the cross-sectional

area of the ommatidium than in bees and hornets (see Figures 4 and 6). The microtubules of the rhabdomeres have a complex organization not seen in the bee or wasp. The long axes of the microtubules appear to lie in three planes, only one of which parallels the cross section of the ommatidia. The microtubules reveal a complexity of structure, such as electron dense areas which appear in the spaces between adjacent tubules and in the center of each of the microtubules.

Hornet. The bald-faced hornet (Vespa maculata) has a typical apposition compound eye. The crystalline cone contains four cellular segments which continue as tubular extensions to the basement membrane. These cone cell extensions and the desmosomes binding adjacent retinula cells are illustrated in Figure 6a. The rhabdom is of the fused type containing four rhabdomeres elaborated by eight retinula cells (Figure 6b). Occasionally an ommatidium containing nine retinula cells has been observed. The general structure of the hornet eye closely resembles that described for the honeybee. Cytoplasmic "fingers" from the surrounding pigment cells protrude between the retinula cells to end in synaptic-like terminals on the crystalline cone cell extensions. This structural complex may serve as a link in information transmission related to pigment migration during light and dark adaptation.

Firefly. The firefly (Photuris pennsylvanica) has a superposition type ommatidium. Much of the ommatidial length is occupied by the clear cells which make up the crystalline cone. The rhabdom extends

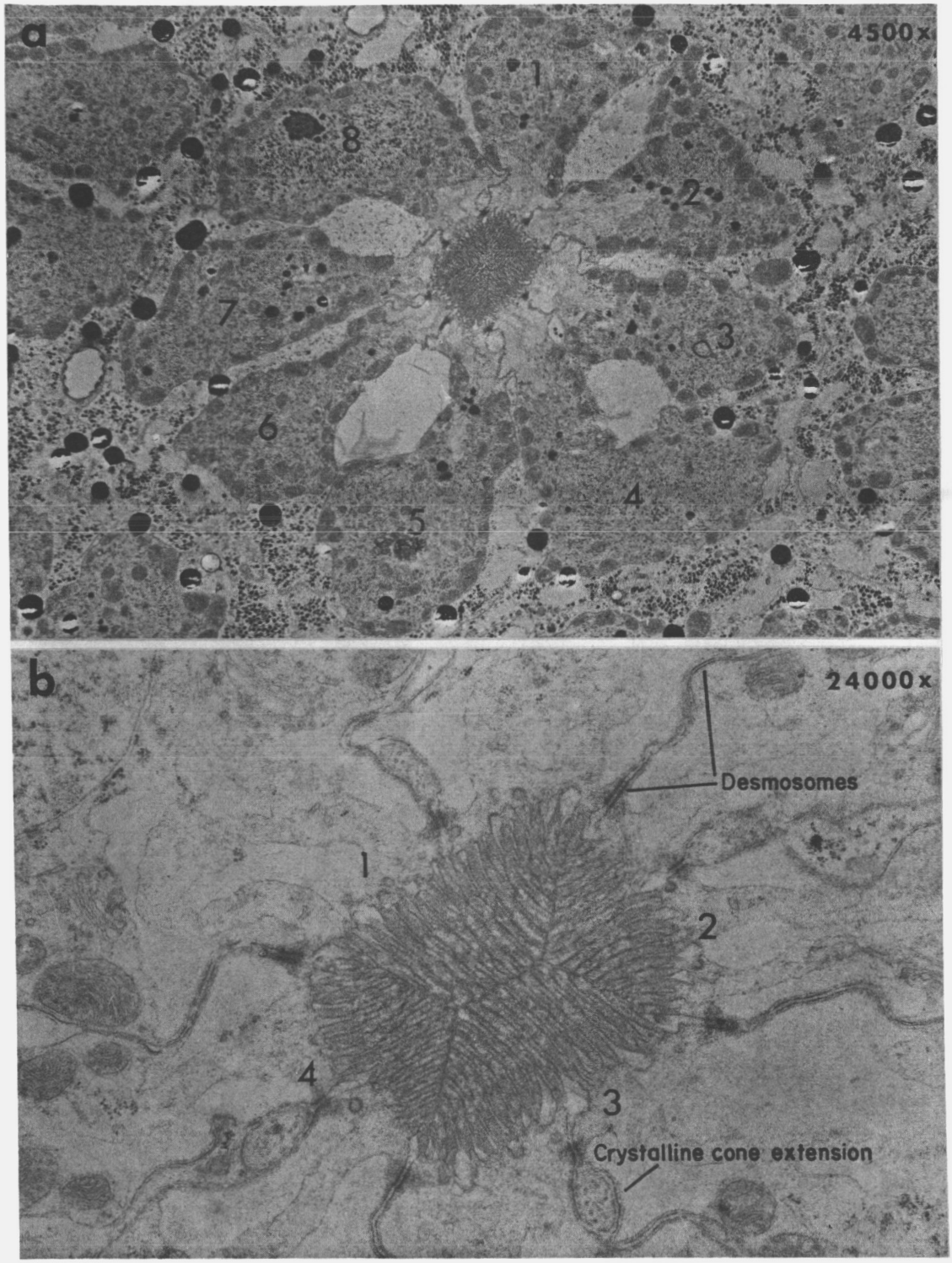


Figure 6 The hornet rhabdom, cross-section: a, the retinula cells (1-8); b, the fused rhabdomeres (1-4).

over less than one fourth of the ommatidial length. However, it occupies nearly the entire cross-sectional area of the ommatidium with only small amounts of retinula cell cytoplasm visible in the region of the rhabdom. There are apparently six cylindrically arranged retinula cells with nuclei located either above or below the rhabdom area. Each of the retinula cells has a V-shaped rhabdomere. The rhabdomeres of adjacent cells are fused to give a rhabdom with the pattern of a six-spoked wheel. The rhabdomeres have the microtubular fine structure characteristic of all insect photoreceptors. There is a dense layer of basal pigment granules concentrated near the basement membrane. This is fenestrated, and axonal processes from the retinula cells pass through these openings in the basement membrane to end in complex synapses on nerve cells of the lamina ganglionaris.

Clothes Moth. The clothes moth (Tineola bisselliella) has a typical superposition ommatidium. The crystalline cone contains four wedge-shaped sectors which are produced by four crystalline cone cells and are surrounded by the distal pigment granules of the system. The area between the proximal end of the crystalline cone and the distal surface of the rhabdom is filled with clear cells. The rhabdom is composed of six fused rhabdomeres. Longitudinally orientated tracheols begin above the rhabdome and surround each ommatidium in a hexagonal pattern. These structures are dumbbell-shaped, have a birefringent center and present a similar architecture to the giant tropical moth (Erebius odora). The tracheols continue below the

rhabdom where they interdigitate with a labyrinth of the tracheol tapetum which is characteristic of nocturnal insects. This reflecting tapetum serves to reflect light back to the rhabdom area. The basal pigment granules are found immediately below the tracheol tapetum.

3. Electrophysiology of the Compound Eye (Beals and Reitboeck)

Information Transfer. Studies on lateral inhibition and the temporal aspects of information transfer are presently being performed. Glass microelectrodes (tip diameter=<1.0μ) filled with 3M KCl are employed to detect responses of single neurons in the visual system of insects. Fiber optics are utilized to confine the light stimulus to well defined areas of the eye.

In the cockroach (Periplaneta americana) action potentials from individual neurons have been detected in all regions of the visual system except the retinula layer. Only gross d.c. potentials (electroretinograms) have been observed in the latter. The failure of the present investigators to observe spikes in the retinula layer of the cockroach supports the growing concept that the retinula layer of most, if not all, insect compound eyes exhibit only graded responses. Since the probability of impaling individual retinula cells of cockroach compound eyes is very low, the study of information transfer in the primary receptors of the visual system will be performed on other species of invertebrates.

Spectral Sensitivity of Periplaneta americana. Since it is important

to carry out pigment extraction at wavelengths which do not influence pigment migration, experiments were performed to determine the wavelengths to which the compound eye responds. The amplitude of the electroretinogram was employed as an index of response to monochromatic light. A Bausch and Lomb monochromator was used to deliver the light stimulus. Responses were observed to light ranging from 400 to 625mμ. Although there have been no reports of response to wavelengths above 600mμ, some evidence for the "influence" of longer wavelengths has been presented. The results of this investigation support the previous evidence for the influence of wavelengths greater than 600mμ and indicate that pigment extraction should be carried out at wavelengths greater than 625mμ.

4. A Novel Crustacean Eye Optical System
(Florida and Wolken)

The copepod Copilia, a marine crustacean which is found in the Mediterranean and Caribbean seas, is of considerable interest in exploring the optical system of arthropods. It is believed that the Copilia eye can scan the environment segment by segment. The Copilia eyes (ocelli) are considered primitive in the respect that its field scanning mode is rather inefficient (i.e., slow), but it has adapted to low light levels with a comparatively "advanced" optical system. The eye consists of a double lens system (Figure 7), where the secondary lens "crystalline cone" (L_2) is placed close to the rhabdomeres and inside the focus of the corneal lens (L_1). Calculations based on our data reveal that the "speed" of the corneal lens is

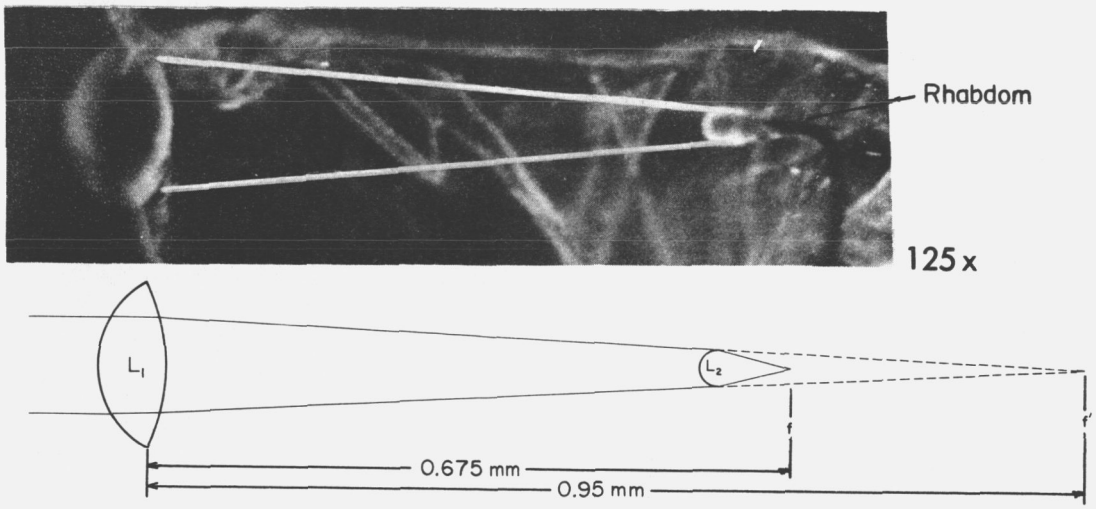


Figure 7 Optics of the Copilia quadrata eye.

increased from a focal ratio of 5.5:1 to 2.5:1 or an increase in the light gathering efficiency by five times. There is a decrease in image size along with this because the effective focal length is reduced, but the decrease in image size is not as significant as the increase in image intensity. The image intensity increases inversely as the area, whereas the resolution decreases linearly with image size.

5. Insect Visual Pigments

Further Studies of a Photosensitive Pigment from the Housefly (Marak, Cornesky, Gallik and Wolken). Although several insect visual pigments have been isolated, e.g., the cockroach and the bee, their properties and how they fit into the scheme of visual biochemistry are not completely understood. The honeybee visual pigment with a 440mμ major absorption peak is unique among all visual pigments in

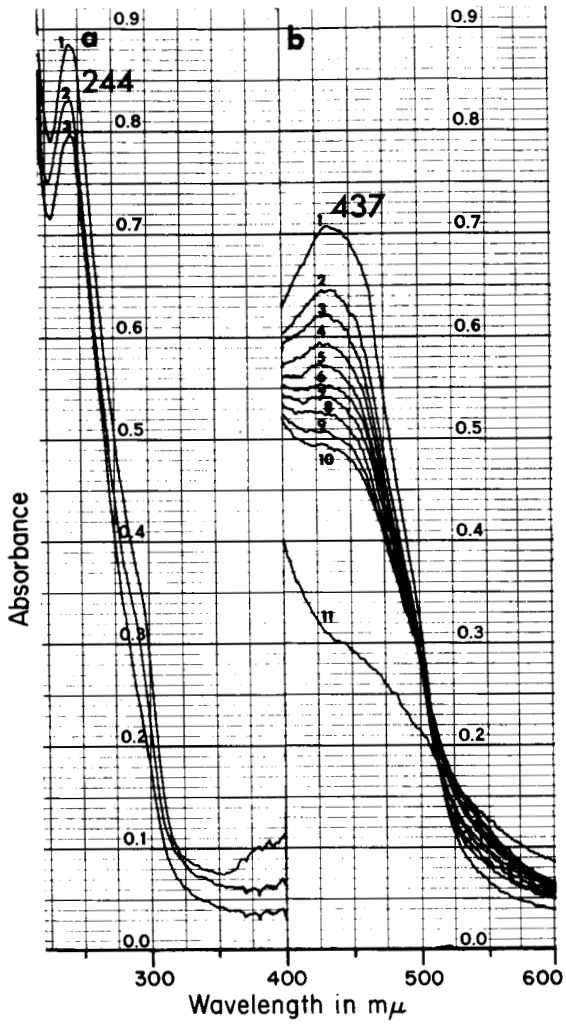


Figure 8 Housefly (Musca domestica) photosensitive pigment. (a) 1:10 dilution of (b)

that it is soluble in aqueous phosphate buffer solutions without use of detergents to solubilize the complex.

In this study large quantities of a light sensitive pigment were obtained from housefly heads by enzymatic degradation of the eye tissues. This material was separated and purified by polyacrylamide zone electrophoresis and gel diffusion chromatography. The isolated light sensitive yellow pigment migrates as a single band on electrophoresis. It has indicator properties, yellow at neutral pH, turning pink at pH5 and colorless above pH8. The pigment was light sensitive from pHs 2-14 but was light stable below pH2). The bleaching by light proceeded at equal rates at 20°C and 5°C. The bleaching spectra shows a loss of absorption at both 437mμ and 244mμ (Figure 8). This confirms our previous data: Bowness, J.M. and Wolken, J.J., J. Gen. Physiol. 42:779, 1959 and Wolken, J.J., Bowness, J.M., and Sch er, I.J., Biochim. et Biophys.

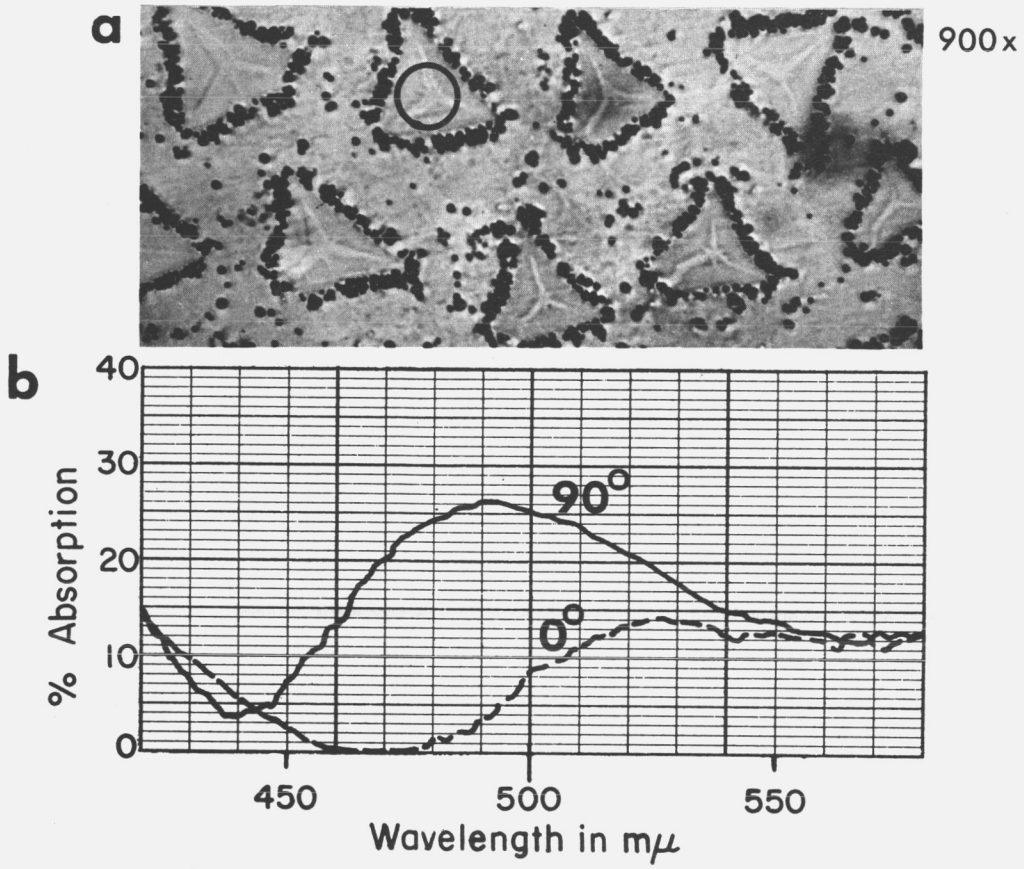


Figure 9 a, Cockroach (Blaberus giganteus) rhabdoms; b, spectra of a single rhabdom with polarized light. (Microspectrophotometer M-5)

Acta. 43:531, 1960. Antimony tests used to determine the presence of vitamin A or retinal in the light sensitive pigment were negative.

Honeybee (Marak and Gallik). We were also able to extract and isolate from honeybee heads a light sensitive yellow pigment which is similar in behavior to the light sensitive housefly pigment. This light sensitive honeybee pigment spectrum also resembles the rhodopsin-like visual pigment. Previous descriptions of the properties of this honeybee visual pigment were calculated difference

spectra so that a direct comparison at present was not possible. Our findings of a light sensitive ommochrome-like pigment in houseflies as well as a similar pigment isolated from the honeybees, and the microspectrophotometry of the cockroach rhabdom (Figure 9), have raised questions as to whether there is sufficient evidence at present to say that these are the insect visual pigments and as to whether these visual pigments of insects are unique in their solubility in buffer solutions.

B. Related Studies

1. Chloroplastin: an Extract from the Chloroplasts of Plant Cells (Wolken)

A chlorophyll protein complex, chloroplastin, which was extracted by 1.8% recrystallized digitonin from the chloroplasts of Euglena gracilis showed photochemical activity (refer to absorption spectra, Figures 11 and 12, and compare these to Figure 10 for chlorophyll a). This complex was found to be not a simple system, but a very complex one, for not only were the chlorophylls extracted, but also carotenoids, lipids, cytochrome and protein. It was postulated that, because these components of the chloroplast are associated in the right molecular orientation in the quasi-crystalline matrix of the digitonin micelle similar to the chloroplast molecular packing, functional aspects of this complex were obtained. New attempts have since been made to isolate different pigment fractions from chloroplasts (Euglena and spinach) using 1.0% digitonin to see which, if possible, of the one or more chlorophyll fractions contain the

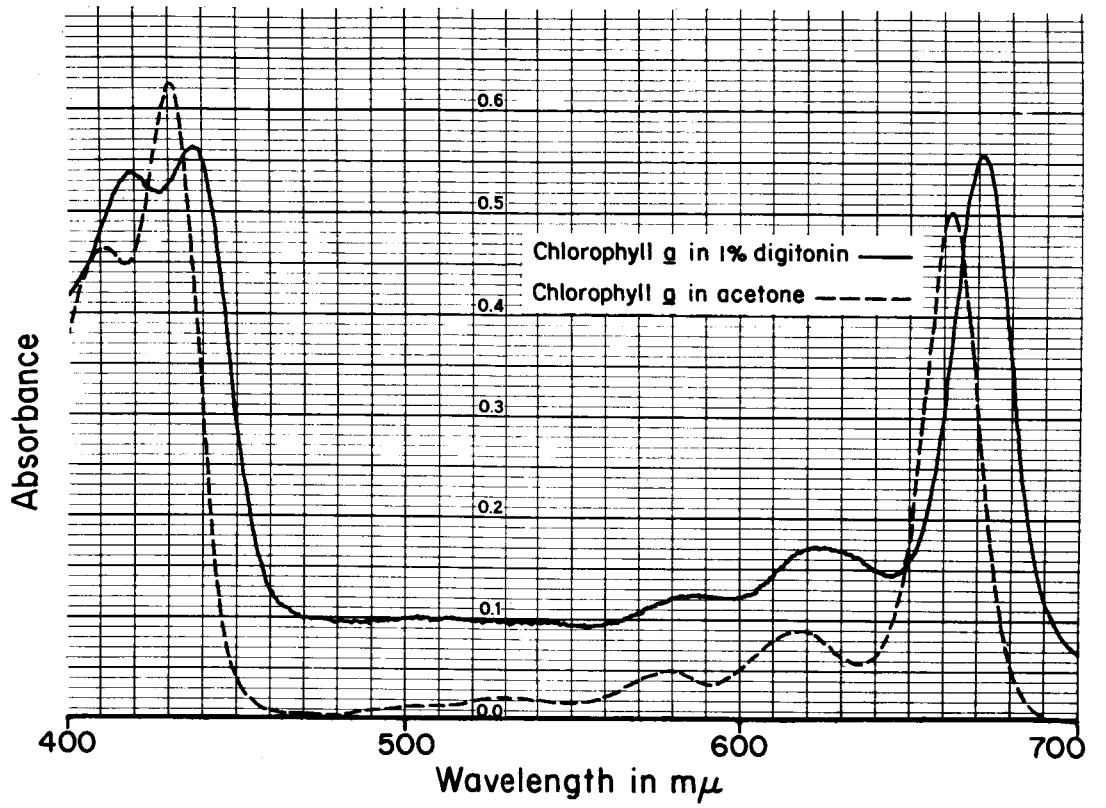


Figure 10 Spectra of chlorophyll a.

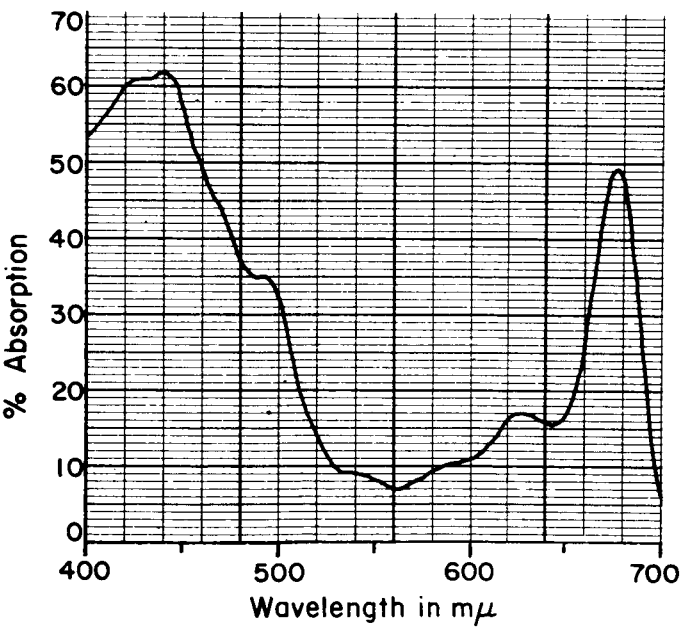


Figure 11 Extract of chloroplasts from Euglena in 1% digitonin. (Microspectrophotometer M-5)

functional components. Attempts were also made to see which, if any, of these pigment complexes are consistant with the two chlorophyll pigment systems hypothesized for photosynthesis. Of the pigment fractions isolated, two are chlorophyll containing and others are carotenoids. These were isolated by chromatography, density gradient ultracentrifugation and gel electrophoresis. They differ in pigment concentration and pigment ratios. These methods, their spectra and other physical and biochemical studies applied to the isolated chlorophyll fractions were done in order to better visualize the arrangement of the molecules within the chloroplast for function in photosynthesis.

2. Effects of Radio-Frequency and Magnetic Fields on the Growth of Euglena gracilis Z. (Vora, Davis and Wolken)

To determine whether exposure of micro-organisms to magnetic and radio fields has any measurable effects or mutations on growth or pigment synthesis, cultures of Euglena in RF and magnetic fields were studied. The oscillation frequency of the RF field was 4.2 Mc/sec. and the peak electric field between the RF plates was 160 volts/cm. Small aliquotes of experimental and control cultures were taken at 24-hour intervals for cell counts, structural observations and biochemical assays. Cell counts and total chlorophyll determinations show an increase in the growth rate of the experimentally RF irradiated over control cultures. Studies of Euglena in pulsed RF fields have shown cellular alignment in the field and an increase in their swimming rates. These radiated RF Euglena cells show, by

interference microscopy, a rearrangement of their chloroplasts within the cells. In addition, these chloroplasts appear to be more refractile than those found in non-irradiated cells. A study to determine whether RF energy produces any change in the chlorophyll molecule, any structural reorganization of the chloroplast or any differences in enzymatic activity is presently in progress.

C. Instrumentation

1. Microspectrophotometry
(Wolken, Forsberg, Florida and Gallik)

The recording Microspectrophotometer M-5 developed over the past two years in our Laboratory is a new instrument designed to extend the usefulness of our earlier models M-1, M-2, M-3 and M-4. The design, performance and application of these instruments to study pigments and the biosynthesis of pigments and other organic molecules within a living cell of a variety of plant and animal tissue cells has been presented in various published papers, in reports at scientific meetings and in NASA reports. The M-5 improvements include better optical resolution; increased sensitivity, especially in the ultraviolet, as well as the visible and the near infrared; operation at low light levels (10^4 photons per second or a total of 2×10^5 photons for a measurement); and reduction of the noise in the entire system.

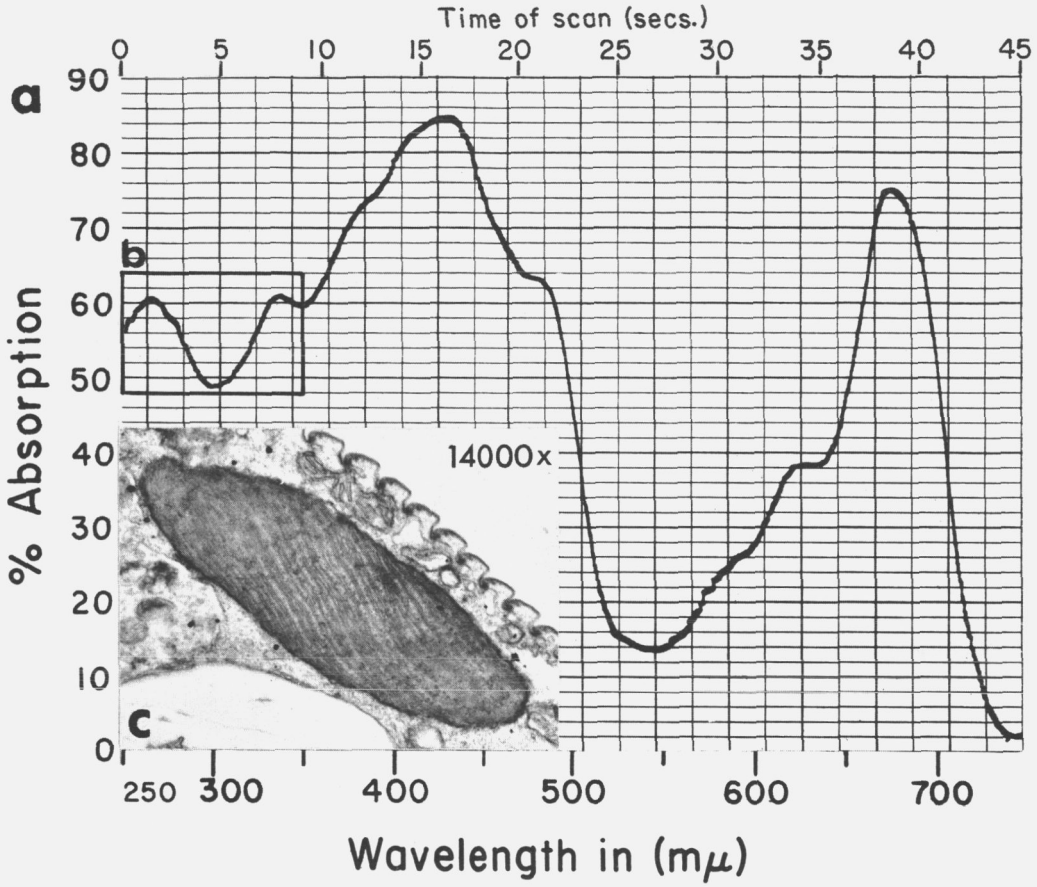
The Microspectrophotometer M-5 was designed to (1) Study the biosynthesis of pigments in the living state, e.g., chlorophylls, carotenoids, porphyrins, cytochromes, hemes. All of these are

important organic molecules involved in energy transfer and necessary for life; (2) Detect changes in single red blood cells which are important for the identification of various blood diseases; (3) Follow the processes of visual excitation in single rods and cones in the retina of the eye; and (4) Identify organic molecules in fossils, minerals and extraterrestrial debris.

Examples of Spectra. Particular emphasis is being directed toward the identification of pigment molecules, e.g., porphyrins, chlorophylls, hemoglobins, carotenoids. These pigments are directly related to the energetics of living cells; and therefore, the ability to identify and follow their synthesis has much to tell us about these kinds of organic molecules in the life processes and to answer questions regarding the evolution of life itself.

Figure 12 is an example of the absorption spectrum from the chloroplast in a living Euglena cell from the ultraviolet to the infrared in a single sweep. This spectrum is similar in peak position and height to the spectrum of chlorophyll a (Figure 10). Figure 12 also shows the ability of the instrument to expand the sensitivity in the ultraviolet bands from 2500 Å to 3500 Å, which shows absorption bands near 2700 Å for proteins and near 3400 Å, probably lipids. Compare this spectrum to Figure 11 for chloroplastin, which shows that a direct comparison can be made on the same instrument.

Studies of the retinal photoreceptors, the rods and cones of the eye, indicate that microspectrophotometry is most useful in studying the visual pigments (i.e., the photochemistry of the



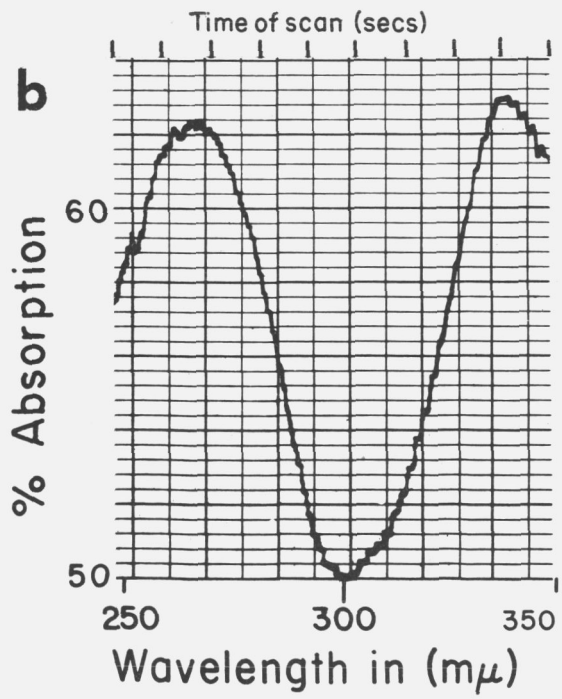


Figure 12 a, Spectrum of a chloroplast of Euglena; b, with the uv portion expanded. (Microspectrophotometer M-5) c, e.m. of Euglena chloroplast.

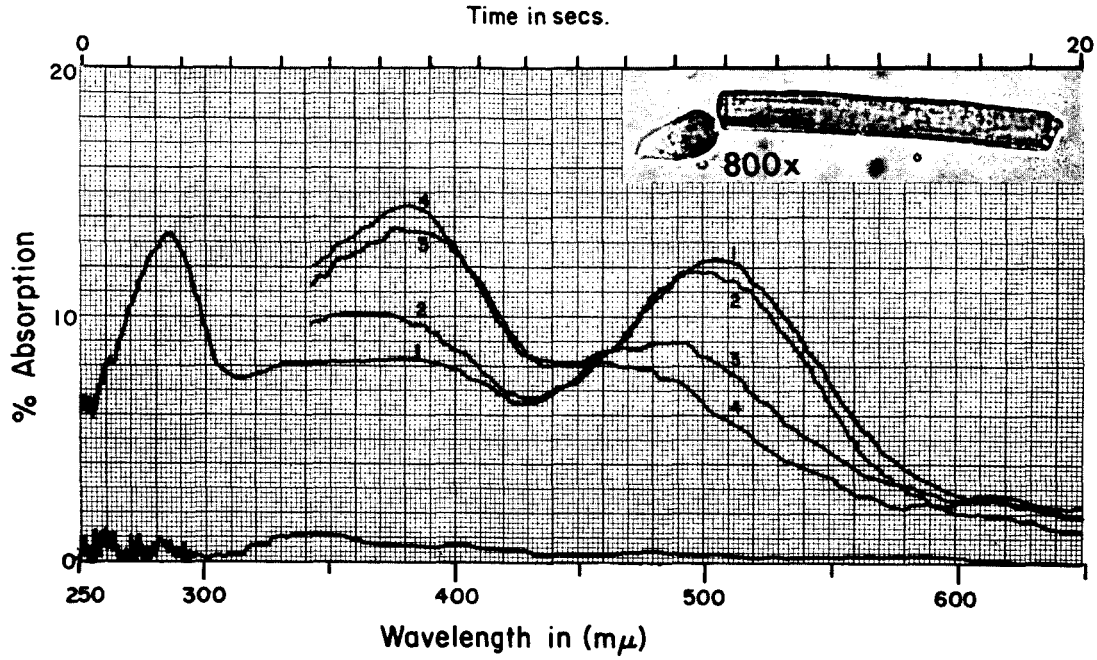


Figure 13 Bleaching spectra of an isolated frog rod outer segment (from Rana pipiens). Curve 1 is unbleached and includes the uv portion of the spectrum. Curves 2, 3 and 4 show the spectrum after bleaching for 15, 30 and 45 seconds at 5000 Å. (Microspectrophotometer M-5) Insert; isolated frog rod.

visual process during light $\rightleftharpoons$ dark reactions). The principal component of the retinal rod is the visual pigment, rhodopsin. It accounts for about 40% of the dry weight of the frog retinal rod outer segment. A typical spectrum from 2500 Å to 6500 Å for a frog retinal rod outer segment is illustrated in Figure 13. The curve shows absorption peaks near 2800, 3500 and 5000 Å, which is typical of the visual pigment-proteins, rhodopsins. The light-bleached spectra, curves 2, 3 and 4 show the increase of absorption around 3800 Å ($retinal_1$) with the loss of absorption of rhodopsin at 5000 Å. The protein peak near 2800 Å does not change with light bleaching.

Application of Microspectrophotometer M-5 by Visiting Scientists. Professor Sidney Fox and Richard Roberts (Institute of Molecular Evolution, University of Miami, Miami, Florida) were interested in obtaining information regarding the absorption spectra, concentrations and DNA incorporation into laboratory synthesized microspheres, which they believe are precursor structures in the processes toward the evolution of life.

Professor George Mueller, also of the Institute of Molecular Evolution, examined samples of foxite from a mineral vein, Derbyshire, England; ozocerite; a film extracted from quartz found in S.W. Africa; and microsphere-like inclusions in this S.W. African quartz.

Dr. Robert Feller (Mellon Institute, Pittsburgh, Pennsylvania and National Gallery of Art, Washington, D.C.) searched for the identity of paint particles obtained from great works of art in order to compare these with newer synthetic paints and determine the legitimacy of these works of art.

Dr. C. L. Stevens and Edgar L. Dimmick (Biophysics Department, University of Pittsburgh, Pittsburgh, Pennsylvania) searched for possible liquid crystalline structures in the liquid phase of polyadenylic acid.

D. Student Research Projects

Auerbach, A.: Lipid analysis of bovine brain and optic nerve. Ellis, N.: Studies of swimming rates of Euglena gracilis as influenced by radio-frequency field. Horn, C.: Extraction of flagella proteins from Euglena gracilis. Hornick, J. and Tumpson, D.: Effects

of radio-frequency fields on insect color preference. Kupcik, M.L. and Wolken, J.: Investigation of a possible two channel analyzer in the insect rhabdom for the detection of plane polarized light. Lubiniecki, A.: Function of prodigiosin in the metabolic pathway of the pigmented bacterium, Serratia marcesens. Miller, J.: Electroretinograms on the cockroach after stimulation with monochromatic light. Neil, B.: Molecular weight determination of mammalian visual pigments. Reducha, P., Winans, J. and Kogut, D.: Isolation, purification and characterization of insect photopigments by chromatography, and analytical and prep disc electrophoresis.

IV. THE LABORATORY

A. Facilities Available

The Biophysical Research Laboratory occupies 5,000 square feet of well equipped laboratories - for biochemistry, biophysics, electrophysiology, electron microscopy, microbiology, magnetics, histology, physical chemistry, electron optics and tissue culture. These research laboratories include equipment for performing preparative and analytical ultracentrifugation, electron microscopy, spectroscopy, electrophoresis, radiation, magnetics and electronics. In addition, there is an experimental instrument shop, photographic darkrooms, greenhouse, specialized library and offices.

The Laboratory staff also has available the collection of the Hunt Botanical Library, Carnegie-Mellon University, the collections of the Carnegie Museum, the Phipps Conservatory for botanical material and the clinical laboratories of Eye and Ear Hospital for cooperative research.

B. Educational Aspects

The Biophysical Research Laboratory has tried to play a part in the development of a new Department of Biological Sciences. The Laboratory affords research opportunities for a number of graduate students and research fellows. It also serves for interdisciplinary training and research with other departments in Carnegie Institute

of Technology and with the Committee on Biotechnology. In addition, graduate teaching in the Biological Sciences profits from the Biophysical Research Laboratory. The close geographic proximity of Carnegie-Mellon University to the Eye and Ear Hospital, the University of Pittsburgh Medical Center Hospitals and the University of Pittsburgh makes it possible to continue cooperative research programs and graduate training in vision, biophysics and physiology.

Dr. Wolken has organized seminars and tutorials in close association with the research of the Biophysical Research Laboratory. He is also teaching a course, Science Systems, to non-science majors. Mr. Cornesky has been teaching a course in Immunology; Dr. Marak has supervised undergraduate research topics; Mr. Gallik and Research Fellows have supervised training in modern research methods; and Mr. Davis has supervised research projects for pre-college students. All of these efforts have gone toward the strengthening of the Biological Sciences at Carnegie-Mellon University. Hopefully through these programs, an interest in basic research will be fostered, and the tools and environment of the Laboratory will help develop initiative toward creative research at a crucial stage in the development of scientific careers.

Opportunities and encouragement are given to the research staff of the Laboratory to work at other research laboratories or universities for periods of time to broaden their own scope.

Since the Laboratory was established, it has been a policy to grant postdoctoral research fellowships. In addition, funds and facilities for visiting research scholars and medical students have

been provided by the Scaife family. These fellows and scholars help provide the stimulating environment so necessary for productive research.

The Biophysical Research Laboratory staff has participated through seminars in other departments of Carnegie Institute of Technology as well as in the College of Fine Arts. In addition, the Laboratory has provided lectures and facilities to various science programs for high school students on the Carnegie campus. Members of the Laboratory staff have also contributed reports of their research to various scientific societies and to the Community.

V. PUBLICATIONS

Wolken, J. J. (1967) Euglena: An Experimental Organism for Biochemical and Biophysical Studies, 2nd edition. Appleton-Century-Crofts, New York.

Wolken, J. J. (1967) The Chloroplast, in The Growth and Development of the Chloroplast to Photosynthesis, edited by C. Sirvonal. Edition Masson, Paris, France.

Wolken, J. J. (1967) The Eye: The Retina, in The Encyclopedia of the Biological Sciences, edited by Peter Gray. Reinhold Publishing Company, New York.

Wolken, J. J. (1967) The Eye of the Crustacean, Copilia. J. Gen. Physiol. 50, 2481.

Wolken, J. J. (1968) Photobiology. Reinhold, New York.

Wolken, J. J. (1968) The Cell and the Red Blood Cell, in First International Conference on Hemorheology. Pergamon Press, Oxford, England.

Wolken, J. J. (1968) Cellular Organelles and Lipids. J. Am. Oil Chem. Soc., April.

Wolken, J. J. (in press, 1968) The Photoreceptors of Arthropod Eyes. London Zoological Society, London, England.

Beals, Barbara S. and Boyarsky, L. L. (in press, 1968) Analysis of Evoked Motor Unit Activity by Auto- and Cross-Correlation. J. Exp. Neurology.

Wolken, J. J., Forsberg, R., Gallik, G. J., and Florida, R. G. (submitted for publication, 1968) A Rapidly Recording Microspectrophotometer. Rev. Sci. Instr.

"Vision: Against the Dark, Part II," The Laboratory, 36:1-6, 1968, H. Schwalb, ed.

Reprints of published papers, when available, will be sent upon request.

VI. REPORTS OF RESEARCH TO SCIENTIFIC MEETINGS AND INVITED LECTURES

Wolken, J. J., January 10, 1967, Research in Photobiology, PENNSYLVANIA JUNIOR ACADEMY OF SCIENCE, Mt. Mercy College, Pittsburgh, Pennsylvania.

Wolken, J. J., February 10, 1967, Photo-Processes of Living Cells, Pt. I, CENTER FOR THEORETICAL STUDIES, UNIVERSITY OF MIAMI, Coral Gables, Florida.

Wolken, J. J., February 13, 1967, Photo-Processes of Living Cells, Pt. II, CENTER FOR THEORETICAL STUDIES, UNIVERSITY OF MIAMI, Coral Gables, Florida.

Wolken, J. J., February 16-17, 1967, Comparative Structure of Photoreceptors, SEMINAR ON PHOTOBIOLOGY, Smithsonian Institution, Washington, D. C.

Wolken, J. J., Gallik, G. J., and Cornesky, R. A., February 22-25, 1967, Purification of Cattle Rhodopsin by Immunochemical Techniques, BIOPHYSICAL SOCIETY MEETINGS, Houston, Texas.

Wolken, J. J., April 5, 1967, Photo-Processes in Biology, Seminar at WEST VIRGINIA UNIVERSITY, Morgantown, West Virginia.

Wolken, J. J., May 7-10, 1967, Cellular Organelles and Lipids, LIPID MONOLAYER AND BILAYER MODELS AND CELLULAR MEMBRANES SYMPOSIUM, American Oil Chemists Society, New Orleans, Louisiana.

Wolken, J. J., May 11, 1967, From Thomas Huxley to Molecular Biology, SIGMA XI, at DUQUESNE UNIVERSITY, Pittsburgh, Pennsylvania.

Wolken, J. J., May 30, 1967, The Photoreceptors of Arthropod Eyes, SYMPOSIUM ON INVERTEBRATE RECEPTORS, London Zoological Society, London, England.

Wolken, J. J. and Gallik, G. J., June 9-10, 1967, Vision Research - Invertebrates, FOURTH ANNUAL MEETING (International), CENTER FOR VISUAL SCIENCE, University of Rochester, Rochester, New York.

Wolken, J. J., Vora, M. R. and Ahn, K. S., June 28-30, 1967, Biochemical Studies of the Flagella of Euglena gracilis Z., the 20TH ANNUAL MEETING OF THE SOCIETY OF PROTOZOOLOGISTS, Toronto, Canada.

Wolken, J. J., July 27, 1967, Comparative Aspects of Photobiology, WOODS HOLE OCEANOGRAPHIC INSTITUTION, Woods Hole, Massachusetts.

Wolken, J. J., August 29-31, 1967, The Eye of the Crustacean, Copilia, 22ND ANNUAL MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS, Woods Hole, Massachusetts.

Marak, G. E., September 30, 1967, Research in the Biophysical Research Laboratory, PENNSYLVANIA LIONS CLUB, Pittsburgh, Pennsylvania.

Wolken, J. J., December 2, 6, 13, 18, 1967, course of lectures in photobiology, UNIVERSITY OF PARIS, SCHOOL OF MEDICINE, Paris, France.

Wolken, J. J., December 4, 1967, The Chloroplast Structure and Photosynthesis, Seminar at CENTER FOR NATIONAL RESEARCH, LABORATORY OF PHOTOSYNTHESIS, Gif-sur-Yvette, France.

Wolken, J. J., January 4, 1968, Photoreception and the Visual Apparatus, CENTER FOR ATOMIC ENERGY, Saclay, France.

Wolken, J. J., January 11, 1968, Research on Photoreceptors, CENTER FOR NATIONAL RESEARCH, NEUROPHYSIOLOGICAL INSTITUTE, Marseille, France.

Vora, M. R., Davis, W. R. and Wolken, J. J., February 18-21, 1968, Effect of Radio-Frequency and Magnetic Field on the Growth of Euglena gracilis Z., 12TH ANNUAL MEETING OF THE BIOPHYSICAL SOCIETY, Pittsburgh, Pennsylvania.

Wolken, J. J., February 28, 1968, Optical Systems of Animals for Vision, OPTICAL SOCIETY OF AMERICA, PITTSBURGH SECTION, Pittsburgh, Pennsylvania.

Wolken, J. J., May 1, 1968, Optical Systems of Animals for Vision and Engineering, ENGINEERING LECTURE SERIES, UNIVERSITY OF TEXAS, Austin, Texas.

VII. ACKNOWLEDGMENTS

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March 25, 1968

DATE

Jerome J. Wolken

JEROME J. WOLKEN